Acta Derm Venereol (Stockh) 1992; 72: 253–255

A Defective Purine Nucleotide Synthesis Pathway in Psoriatic Patients

R. KIEHL and G. IONESCU

Research Department, Spezialklinik Neukirchen, Neukirchen, Germany

Purine nucleotide concentrations in skin- and bloodcells of psoriatic patients are abnormal: The increase in the steady state level of cGMP and the decrease in the cAMP concentrations are associated with an enhanced rate of cellular proliferation. Concomitantly we found in the present study decreased ADP and ATP concentrations in blood cells ($p < 0.0001$). The change in nucleotide concentrations suggests a defective purine nucleotide synthesis pathway. Stimulation of the Krebs cycle with fumaric acid raises ATP ($p < 0.0001$) and most probably cAMP levels and at the same time slows down the purine nucleotide synthesis through end-product inhibition. Both effects can inhibit DNA and protein synthesis activity, which results in inhibition of cellular proliferation. Fumaric acid seems therefore a useful treatment for psoriatic lesions if liver and kidney functions (purine nucleotide and urea cycle) are controlled during treatment. ***Key words: Fumaric acid: DNA synthesis; Protein synthesis.***

(Accepted December 16, 1991.)

Acta Derm Venereol (Stockh) 1992; 72: 253–255.

R. Kiehl, Research Department, Spezialklinik Neukirchen, D-8497 Neukirchen, Germany.

More than 30 years have passed since the biochemist Schweckendick in 1959 (1) reported successful treatment of his own psoriatic lesions with fumaric acid. Since then more and more psoriatic patients have been satisfied with fumaric acid therapy, although some physicians are still sceptical about this approach (2). Schweckendick (1, 3) and Schäfer (4, 5) emphasize that fumaric acid is an intermediate in the Krebs cycle. Dubiel & Happle (6) found with high dosages of fumaric acid in 5 out of 6 patients an anti-psoriatic activity accompanied by pathological kidney parameters.

To find a plausible explanation for the effect reported after treatment with fumaric acid we decided to investigate the related purine nucleotide levels in the blood cells of psoriatic patients and healthy controls.

PATIENTS AND METHODS

Twenty patients (age range 19–52 years) with clinically proved psoriasis vulgaris (7) and more than a 5-year disease history as well as 13 healthy volunteers having no history or signs of a skin disease (age range 21–46 years) agreed to participate. The patients avoided all steroid and/or phototherapy for at least 2 months.

ATP- and ADP-concentrations in sera and whole blood of patients and controls were determined with the ATP monitoring kit (1243-102) from Colora Meßtechnik GmbH, Germany, using the bioluminescence technique in a LKB 1214 Rackbeta liquid scintillation counter. The lyophilized ATP-monitoring reagent and the ATP-standard were reconstituted in 10 ml of double distilled, deionized water each.

1 ml fresh EDTA-blood or 1 ml fresh serum was shaken vigorously in 4 ml ice-cold trichloracetic acid solution (1.2 M), after 10 min incubation at room temperature the mixture was centrifuged for 10 min at 3000 rpm and the resulting supernatant frozen at −20°C until measurements were performed (not more than 6 weeks later).

A) *ATP:* 10 µl sample (supernatant EDTA-blood or serum) together with 40 µl ATP monitoring reagent mixed into 2 ml 40 mM Na-Hepes-buffer, pH 7.75, was measured after 1 min in the scintillation counter to get ATP concentration. An ATP-standard curve has been obtained with appropriate amounts of ATP-standard solutions. The level of detection is 10 to 20 nM ATP.

B) *ATP + ADP:* 10 µl sample (supernatant EDTA-blood or serum) was incubated with 10 µl phosphoenolpyruvate solution (100 mg/ml, Boehringer Mannheim) and 10 µl pyruvate kinase (400 U/ml, Boehringer Mannheim) in 2 ml 40 mM Na-Hepes-buffer, pH 7.75, for 2 min; then 40 µl ATP monitoring reagent was added and the nucleotide concentration was measured after 1 min in the scintillation counter.

Serum ADP could not be estimated because phosphoenolpyruvate and pyruvate kinase were not clean enough to allow the detection of the low concentrations present in sera. However, it was possible to calculate that ADP concentrations were in the range of ATP concentrations. cAMP in EDTA-plasma and EDTA-blood was analyzed by RIA in the laboratory of Drs. Hofmeister, 8480 Weiden/FRG.

Eleven patients were treated orally with fumaric acid capsules, potency I and III, according to Schäfer. Capsules potency I contained 30 mg fumaric acid, 20 mg Ca-, 5 mg Fe-, 10 mg Li-, 20 mg Zn-, 1 mg Cu-, 10 mg K- and 20 Mg salts of fumaric acid monoethylester, as well as 30 mg fumaric acid dimethylester. Capsules potency III contained 50 mg Ca-, 6 mg Fe-, 1 mg Cu- and 40 mg Mg salts of fumaric acid monoethylester, as well as 120 mg fumaric acid dimethylester. ATP, ADP + ATP and cAMP measurements in EDTA-blood were performed before and after 2 and 4 weeks of fumaric acid treatment respectively. EDTA-blood samples were taken 2 h after fumaric acid intake and worked out immediately as described above. The patients' liver-, kidney- and haematological parameters were controlled regularly every week during this treatment.

The statistical significance of the results was estimated by means of the Student's *t*-test.

RESULTS

The concentrations of the purine nucleotides cAMP (21 ± 4 nM vs 22 ± 3 nM), ADP (231 ± 83 nM vs 295 ± 140 nM) and ATP (see methods) in the sera of the psoriatic patients when compared to healthy controls were normal, which is contrary

Table I. *ADP-, ATP-concentrations and ATP/(ADP + ATP) ratio in blood of psoriasis patients and healthy controls*

	ATP µM	ATP + ADP µM	ADP µM	ADP/(ADP + ATP)
Psoriasis patients ($n = 20$)	187±64	399±93	212±96	0.47±0.2
Control persons ($n = 13$)	309±74	600±84	292±92	0.52±0.1
Significance Student's *t*-test	$p < 0.01$	$p < 0.0001$	$p < 0.05$	NS

Table II. *ATP and ADP plus ATP levels in blood cells of 11 psoriatic patients in dependence of 4 weeks oral fumaric acid treatment*

	ATP μM	ATP + ADP μM	Fumaric acid mg/day	Fumaric acid monoethylester mg/day	Fumaric acid dimethylester mg/day	Time weeks
Psoriatic patients	170±33	588±117	0	0	0	0
	162±45	536± 88	60	172	60	
	297±64	610±109	90	258	90	2
	386±40	680± 61	0	97	120	
	395±37	655± 82	0	194	240	4
	484±73	714± 79	0	291	360	
Significance Student's *t*-test	betw. 0 and 90 to 360 mg FDME[a]: $p < 0.0001$	change: NS				

[a] FDME = fumaric acid dimethylester.

to the whole blood-nucleotide levels. These levels as well as the ATP/(ADP + ATP) ratio in EDTA-blood and serum of psoriatic patients and healthy controls are shown in Table I. Mean blood ATP level in psoriatic patients was 187 μM vs 309 μM in controls, mean ADP level 212 μM vs 292 μM and mean ADP plus ATP concentrations 399 μM vs 600 μM. The difference was highly significant for ATP plus ADP and significant for ADP or ATP alone. Comparison of the nucleotide concentrations in the sera with whole blood nucleotide levels impressively demonstrates that the change in the described nucleotide concentrations manifests itself entirely in the cell. The ATP/(ADP + ATP) ratio remains unchanged. This constant ATP/(ADP + ATP) ratio makes clear that ATPase/synthetase and PGK (3-phosphoglycerate kinase) are not involved in alterations of the nucleotide levels.

Table II shows ADP plus ATP and ATP concentrations in blood cells of 11 psoriatic patients during the 4 weeks of oral fumaric acid treatment. As expected, the ADP plus ATP concentrations remained unchanged, ATP values increase significantly ($p < 0.0001$) while ADP levels decrease (ATP plus ADP values minus ATP values) according to the increasing fumaric acid dimetylester concentrations. A slow rise to a dosage of once to twice daily one capsule of fumaric acid, potency III, containing 120 to 240 mg fumaric acid dimethylester has proven to be the best medication. Clinically we noticed that the skin of the patients cleared after 4 weeks therapy. There is a clear-cut correlation between raise in ATP concentration and clearing of the skin. A concentration of fumaric acid dimethylester below 100 mg gives only slight improvements of the skin lesions. No changes in the urine and blood parameters were noticed.

DISCUSSION

Wahba and associates (8) postulated that a generalized defect in cyclic nucleotide metabolism may be found not only in epidermal cells but also in neutrophils and monocytes derived from psoriatic patients. In support of this hypothesis, two studies have shown decreased levels of cAMP in psoriatic lymphocytes compared to normal controls (9, 10). We suggest that the former postulate is valid for all purine nucleotides. Beginning with 5-Phosphoribosyl-1-pyrophosphate (PRPP) the purine nucleotides IMP and AMP, ADP, ATP, cAMP were synthesized in one direction and XMP, GMP, GDP, GTP, cGMP in the other direction. ATP and GTP were used in RNA and DNA synthesis, cAMP and cGMP control protein-biosynthesis activity. cAMP levels in psoriatic skin were reported significantly lower than in normal skin, while on the other hand cGMP levels in psoriatic epidermis were higher than in the normal skin (11). The measured cellular concentrations of ADP, ATP, cAMP and cGMP make our suggestion most probable and imply the measurements of the remaining purine nucleotide levels.

Marcello & Duell (12) showed that in adult human epidermis low levels of cAMP stimulated proliferation, whereas high levels inhibited growth. On the other hand, Goldberg & Haddox (13) postulated that an increase in the steady state level of cGMP may be associated with an enhanced rate of cellular proliferation. As has already been mentioned, psoriatic patients express low levels of cAMP, but high levels of cGMP (11). This situation favors then a high rate of cellular proliferation, via acceleration of the protein-biosynthesis activity. One direct way to normalize cellular proliferation is the elevation of the normal fumaric acid concentrations present in the cell by supplementation with oral or external (via skin) fumaric acid (dimethylester) preparations. Fumaric acid will accelerate Krebs cycle, respiratory chain as well as ATPsynthetase and then elevate ATP- and consequently cAMP-levels. Another important result of elevated fumaric acid is its end-product inhibition which slows down purine nucleotide synthesis. Fumaric acid is involved in 3 steps of the purine nucleotide synthesis pathway: a) production of 5-amino-imidazolyl-4-carboxamido-rionucleotide (→ IMP), b) production of AMP and c) production of ATP.

Fumaric acid dimethylester alone can cross membranes. Hydrolysis in mitochondria makes it a substrate for the Krebs cycle. Our results clearly suggest the accelerating power of fumaric acid on ATPsynthetase and the metabolic connection between the different cells. The most important connecting factors are the purines (especially adenosine) and glucose (14–19). We should emphasize that all measured ADP and ATP levels belong mainly to the erythrozytes and are therefore

indirectly controlled by mitochondria and dependent cellular glycolysis. Measuring the actual ADP or ATP concentrations in white blood or tissue cells alone is to our knowledge impossible with the present methods.

A twofold rise in the ATP concentration due to mitochondrial fumarate increase should lead to a raised cellular cAMP production, as mentioned above. Our cAMP assay in whole blood was not sensitive enough to demonstrate this assumption, and further investigations on isolated white blood cells are in progress.

Previous findings (20–23) are compatible with our results. They describe the inhibition of DNA synthesis activity and of cellular proliferation by fumaric acid.

Another way to normalize the lowered cellular ATP concentrations seems to be the supplementation of ATP. The positive results of externally delivered ATP on psoriatic lesion are most likely induced by stimulation of adenylate cyclase and by inhibition of cellular DNA synthesis. However, the effective compound hereby is adenosine (24; Paul, D., personal communication). Adenosine itself emerges mainly from the hydrolysis of supplied ATP in the blood stream and may, unlike ATP, activate the β-receptors and cross the cell membranes. Externally supplied ATP cannot directly raise cellular ATP levels.

The cytotoxic reagent methotrexate, used for the therapy of cancer and psoriatic patients (25), inhibits antagonistically all folic acid dependent reactions, among others purine biosynthesis and pyrimidine nucleotide synthesis (dTTP). RNA- and DNA-synthesis and consequently the cell growth and proliferation become equally impaired. Fumaric acid inhibits in contrast only the growth of the most weak (proliferating) cells.

Topically applied dithranol induces free radical reactions with a lot of cell components, among them it also undergoes molecular intercalation complexes and direct reactions with the DNA-chain, thereby inhibiting the cellular growth. A rise of the ATP concentration is not possible in this case because of the various reactions caused by the compound (26, 27).

To our experience, slowly increasing blood concentrations of fumaric acid formulations offer a valuable treatment for psoriatic patients under controlled conditions.

ACKNOWLEDGEMENT

We thank Prof. D. Paul, Hannover, Dr. G. Kram, Mrs. D. Träger, Mrs. B. Karl, Mrs. E. Kaiser, Drs. Hofmeister, Weiden (for performing the cAMP measurements) and all others who helped to finish this manuscript.

REFERENCES

1. Schweckendick W. Heilung von Psoriasis. Med Monatsschr 1959; 13: 103–104.
2. Raab W. Psoriasis-Behandlung mit Fumarsäure und Fumarsäureestern. Z Hautkr 1984; 59: 671–679.
3. Schweckendick W. Behandlung von Psoriasis mit lipidlöslichen Fumarsäureverbindungen. Medizin heute 1966; 15: 219–220.
4. Schäfer G. Psoriasis mit Fumarsäure beherrschbar. Selecta 1982; 17: 1868–1872.
5. Schäfer G. Fumarsäure hindert die Schuppenflechte. Selecta 1984; 19: 1260–1261.
6. Dubiel W, Happle R. Behandlungsversuch mit Fumarsäuremonoäthylester bei Psoriasis vulgaris. Z Haut-GeschlKr. 1972; 47: 545–550.
7. Psoriasis vulgaris. In: Braun-Falco O, Plewig G, Wolff HH, eds. Dermatologie und Veneroelogie. Berlin–Heidelberg: Springer-Verlag, 1984: 381–393.
8. Wahbe A, Cohen H, Bar-Eli M, Gallily R. Neutrophil chemotaxis in psoriasis. Acta Derm Venereol (Stockh) 1979; 59: 441–445.
9. Geerdink JPM, Bergers M, Van Erp PEJ, Gommans JM, Mier PD, Roelfzema H. Cyclic AMP is decreased in mononuclear leukocytes from psoriasis patients. Br J Dermatol 1980; 103: 107–108.
10. Harpaz S, Fink A, Zuckermann F, Muhammed E. Lymphocyte cyclic nucleotide and prostaglandin content in psoriasis: a preliminary report. Arch Dermatol 1980; 116: 427–428.
11. Royer E, Chaintreul J, Meynadier J, Michel B, Guilhon JJ, de Paulet AC. Cyclic AMP and Cyclic GMP Production in Normal and Psoriatic Epidermis. Dermatologica 1982; 165: 533–543.
12. Marcello CL, Duell EA. Cyclic AMP stimulates and inhibits adult human epidermal cell growth. J Invest Dermatol 1979; 72: 279.
13. Goldberg ND, Haddox MK. Cyclic GMP metabolism and involvement in biological regulation. In: Snell EE, Boxner PD, Meister A, Richardson CC, eds. Annual Review of Biochemistry. Palo Alto, CA: Palo Alto Annual Review, 1977: 823–896.
14. Lajtha LG, Vane JR. Dependence of bone marrow cells on the liver for purine supply. Nature 1958; 182: 191–192.
15. Henderson JF, Le Page GA. Transport of adenosine-8-C^{14} among tissues by blood cells. J Biol Chem 1959; 234: 3219–3223.
16. McManus TJ. Alternate pathway for metabolism: a comparative view. In: Greenwalt TJ, Janneson GA, eds. The human red cell in vitro. New York: Grune and Stratton 1974: 49–63.
17. Kim HD, Zeidler RB, Sallis J, Nicol S, Isaacks RE. Metabolic properties of low ATP erythrocytes of the monotremes. FEBS Lett 1984; 167: 83–87.
18. Kim HD. Is adenosine a second metabolic substrate for human red blood cells? Biochim Biophys Acta 1990; 1036: 113–120.
19. Lüthje J. Extracellular adenine compounds, red blood cells and haemostasis: facts and hypotheses. Blut 1985; 59: 367–374.
20. Speiser P. In: Ergebnisprotokoll zum Arbeitsgespräch "Fumarsäure Therapie" des Deutschen Psoriasis-Bundes am 12. März 1988. Hamburg, 1988.
21. Petres J, Kalkoff KW, Baron D, Geiger R, Kumick J. Der einfluß von Fumarsäuremonoäthylester auf die Nukleinsäure- und Proteinsynthese. Arch Derm Forsch 1975; 251: 295–300.
22. Hagedorn M, Kalkoff KW, Kiefer G, Baron D, Hug J, Petres J. Fumarsäuremonoäthylester: Wirkung auf DNA-Synthese und erste tierexperimentelle Befunde. Arch Derm Res 1975; 254: 67–73.
23. Schroeff JG van der, Ondshoorn C, Nugteren-Huying WM, Ponec M. Inhibitory Effects of Fumaric Acid Derivatives on Cell Proliferation and Differentiation. J Invest Dermatol 1989; 92: 537.
24. Anderson TF, Voofhees JJ. Cyclic nucleotides, In: Roenigh HH, Maibach HJ, eds. Psoriasis. New York, Basel: Marcel Dekker, Inc., 1986: 271–284.
25. Steigleder GK, Schulze H-J. Wie wirkt Methotrexat bei Psoriatikern? Z Haukr 1988; 63: 8–10.
26. Lowe NJ, Breeding J. Anthralin. Arch Dermatol 1981; 117: 698–700.
27. Shroot B, Brown C. Free radicals in skin exposed to dithranol and its derivatives. Arzneim-Forsch/Drug Res 1986; 36: 1253–1255.